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# Multiple biomarkers study in painters in a shipyard in Korea

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**Abstract**

Shipbuilding workers are exposed to a variety of genotoxic compounds including polycyclic aromatic hydrocarbons (PAHs). A limited number of studies have been conducted to evaluate biomarkers related to PAH exposure in painters in the shipyard industry. We examined this in 208 workers recruited from a shipyard located in South Korea. Employees were grouped into three exposure groups: (1) 111 painters using coal tar paints, (2) 70 painters using general paints, and (3) 27 on-site controls using no paints. Levels of urinary 1-hydroxypyrene glucuronide (1-OHPG), as internal dose of PAH exposure, were measured by synchronous fluorescence spectroscopy. Glutathione *S*-transferase (*GST*) *M1* and *T1* genotypes were assessed by a multiplex polymerase chain reaction (PCR)-based method, aromatic-DNA adducts in peripheral white blood cells were measured by $^{32}$P-postlabeling, and glycophorin A (GPA) variant frequencies in red blood cells were assessed by flow cytometry. Information on demographic characteristics, smoking habits, diet, job title and use of personal protective equipment (e.g. respiratory and dermal) were collected by self-administered questionnaire.

Average urinary 1-OHPG levels in coal tar paint (2.24 μmol/mol creatinine) and general paint (1.38 μmol/mol creatinine) users were significantly higher than in on-site controls (0.62 μmol/mol creatinine) ($P < 0.001$). Paint use, irrespective of the type of paints, and smoking (yes/no) were positively associated with urinary 1-OHPG levels, whereas green tea consumption (yes/no) was negatively associated with the 1-OHPG levels. No significant effect in the 1-OHPG levels were observed for the *GSTM1* and *GSTT1* genotypes. Aromatic-DNA adduct levels tended to be higher in coal tar paint users ($P = 0.06$) and painters ($P = 0.07$) compared to on-site controls. No differences in adduct levels were observed, between the two groups of painters,

*Abbreviations:* PAHs, polycyclic aromatic hydrocarbons; 1-OHPG, 1-hydroxypyrene glucuronide; GST, glutathione *S*-transferase; BaP, benzo[a]pyrene; GPA, glycophorin A; SFS, synchronous fluorescence spectroscopy; EGCG, (−)-epigallocatechin gallate

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and the combined group showed greater adduct levels than on-site controls ($P = 0.05$). GPA mutation frequencies measured in 55 individuals with MN heterozygote genotypes were not significantly different among the three exposure groups, and no correlation was observed between urinary 1-OHPG levels and aromatic-DNA adducts or GPA mutation frequency. These results suggest that painters in the shipyard were exposed to significant amounts of PAHs and possibly to other genotoxic aromatic compounds, and that urinary 1-OHPG may be a potential biomarker of PAH exposure in this population.


*Keywords:* Polycyclic aromatic hydrocarbon; Shipyard; 1-Hydroxypyrene glucuronide; Genetic polymorphism; Glutathione *S*-transferases

## 1. Introduction

Painters are exposed to a wide variety of hazardous substances including aromatic hydrocarbons, aliphatic hydrocarbons, ketones, alcohols, and esters in paints, thinners, hardeners, and other painting materials [1]. About 40 different types of paint containing coal tar are used in shipyards in Korea and they account for 13% of all shipyard paints [2].

A meta-analysis of lung cancer showed a significant association between painting exposure and lung cancer mortality [3]. A large cohort study of 57,000 painters also indicated an excess risk of lung cancer mortality [4]. Recently, a painter in a large shipyard diagnosed with lung cancer was compensated for occupational lung cancer related to shipyard painting in Korea [5].

Urinary 1-hydroxypyrene (1-OHP) and the glucuronide conjugate of 1-OHP, 1-hydroxypyrene glucuronide (1-OHPG), have been successfully used in a variety of polycyclic aromatic hydrocarbons (PAHs) exposed populations to estimate internal PAH-dose [6,7]. Since urinary 1-OHPG is the major pyrene metabolite in urine and emits three to five-fold fluorescence compared to 1-OHP, it may provide a more sensitive biomarker for the assessment of low-level exposure to PAHs [8,9].

Glutathione *S*-transferases (GSTs) are a superfamily of enzymes involved in conjugation of reactive intermediates to less reactive and more easily excreted metabolites, and therefore, play an important role in the detoxification of endogenous and exogenous toxicants [10]. For instance, *GSTM1* isoform can detoxify PAHs, such as benzo[a]pyrene (BaP), while *GSTT1* can detoxify smaller reactive hydrocarbons, such as ethylene oxide and diepoxybutane, and is also involved in the metabolism of several solvents [11,12].

The effect of genetic polymorphisms of selected metabolism enzymes like *GSTM1* and *GSTT1* on the level of urinary PAH metabolites has been reported for individuals with PAH exposure in various settings like pot-room workers [13], coke oven workers [14,15] and non-occupationally exposed individuals [16,17]. The effect of *GSTM1* and *GSTT1* genotypes on aromatic-DNA adduct levels in peripheral white blood cells have also been assessed in a number of previous studies, including coke oven workers [18], foundry workers [19], and non-occupationally exposed individuals [20]. However, a limited number of studies have been conducted to evaluate the effects of *GSTM1* and *GSTT1* polymorphisms on the association between aromatic-DNA adducts in peripheral white blood cells and urinary PAH metabolites [21].

The glycophorin A (GPA) mutation assay was developed to quantify the frequency of somatic cell mutations that occur in vivo in human red blood cells [22]. The human GPA gene has two equally prevalent alleles, M and N, whose gene products differ by two amino acids and are the basis for the M and N blood types. The GPA assay uses fluorescent-labeled monoclonal antibodies and erythrocyte flow cytometry to detect the loss of one of these forms from the cell surface [23]. Two variant cell phenotypes are measured with this assay; the hemizygous phenotype (NØ), which lacks expression of one allele and expresses the other allele normally, and the homozygous phenotype (NN), which results from a gene-duplication [23]. However, only a limited number of studies have been published on the usefulness of GPA mutation as an early biological effect marker for genotoxic compounds, such as PAHs [24,25].

In this study, several biomarkers potentially related to PAH exposure were assessed in shipyard paint workers, including urinary 1-OHPG and 1-OHP as internal PAH dose measures, aromatic-DNA adducts in peripheral white blood cells as measures of biologically effective dose, and GPA determined in human

somatic cells as an early marker of biological effect. The effect of *GSTM1* and *GSTT1* genotypes on these biomarkers was also evaluated.

## 2. Materials and methods

### 2.1. Materials

The hybridoma cell line for monoclonal antibody 8E11 (Mab 8E11) was kindly provided by Dr. Regina Santella, Columbia University, New York. 1-OHPG was obtained from the NCI Chemical Carcinogen Repository (MRI, Kansas, MO, USA). The GPA N-specific antibody BRIC 157 and the GPA M-specific antibody 6A7 were obtained from the International Blood Group Reference Laboratory (Bristol, UK). Primers for *GSTM1*, *GSTT1*, and β-globin were purchased from BIONEER (Daejun, Korea). All other chemicals were obtained in the greatest available purity from commercial suppliers.

### 2.2. Study subjects

The study population consisted of 208 workers (191 men, 17 women; mean age 44.6 years old) recruited from a large shipyard located in South Korea. The study subjects were divided into three groups according to the presence of direct exposure to the paint spraying and/or brushing process (Table 1).

Information on demographic characteristics, smoking habits (numbers of cigarettes consumed per day, starting years of cigarettes smoking, nonsmokers were those who had not smoked for past 6 months), alcohol consumption, and diet before the sampling day, and use of personal protective equipment were collected using a self-administered questionnaire.

Table 1
Selected characteristics of study subjects

| Characteristic | Controls | General paint users | Coal tar paint users |
|---|---|---|---|
| *N* | 27 | 70 | 111 |
| Mean age (S.D., year) | 47.4 (6.9) | 43.5 (7.7) | 44.6 (7.9) |
| Male (%) | 89 | 100 | 87 |
| Current smokers (%) | 67 | 74 | 68 |
| Alcohol users (%) | 50 | 63 | 52 |
| Green tea users (%) | 22 | 21 | 26 |
| *GSTM1* null (%) | 44 | 50 | 54 |
| *GSTT1* null (%) | 65 | 49 | 57 |

### 2.3. Sample collection

Personal air samples were collected over 6 h per day for 3 days from the breathing zones of 25 paint spray workers several weeks before the urine samples were collected. Particulates were collected on 37 mm-diameter Teflon filters (SKC, eighty four, PA, USA) in glass sorbent tube containing 100/50 mg XAD-2 resin. After sampling, filters were wrapped in aluminum foil and kept at −20 °C until analysis. Postshift urine samples (50 ml) were collected on Friday in polypropylene tubes and stored at −70 °C until analysis.

At the time of the urine collection, blood samples (5 ml) were collected in EDTA vacutainer tubes and divided into two aliquots. One of the aliquots was used for the GPA assay and was kept at ∼4 °C prior to fixation and analysis, while the other aliquot was stored at −70 °C until used for aromatic-DNA adduct and genotype determination.

### 2.4. Analysis of PAHs in air

Sixteen priority PAHs were measured according to a modified NIOSH method 5515 [26]. Teflon filters and XAD-2 absorbent materials were extracted in 1 ml of dichloromethane by sonication for 30 min, followed by gas chromatography (Hewlett-Packard 5890 series Plus II) with mass selective detector (Hewlett-Packard 5972 MSD) using fluoranthracene as the internal standard. The PAHs analyzed were naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, pyrene, *benzo(ghi)perylene, *benzo(a)anthracene, chrysene, *benzo(b)fluoranthene, *benzo(k)fluroranthene, *benzo(a)pyrene, *indeno(1,2,3-cd)pyrene, and *dibenzo(ah)anthracene (*carcinogenic PAHs) [27].

### 2.5. Analysis of urinary 1-hydroxypyrene glucuronide (1-OHPG)

Urinary 1-OHPG was quantified in urine using the assay developed by Strickland et al. [7]. Briefly, a 4 ml aliquot of each urine sample was treated with 1 N HCl at 90 °C for 1 h to hydrolyze

acid-labile conjugated metabolites and then loaded on methanol/water primed Sep-Pak $C_{18}$ cartridges (Waters, Milford, MA, USA). They were washed with the same volume of water and 30% methanol in water and eluted by the same volume of 80% methanol in water. The eluates were concentrated to about 1/8 of the original volume under vacuum with mild heating, after which the volume was adjusted to 4 ml with 7.5 mM phosphate-buffered saline (PBS) (pH 7.4). The samples were loaded on to immunoaffinity columns (IAC), which contained CNBr-activated Sepharose 4B coupled with monoclonal antibody 8E11 that recognizes several PAH-DNA adducts and metabolites, including 1-OHPG. The columns were washed with 4 ml of 7.5 mM PBS and 25% methanol in 7.5 mM PBS; 1-OHPG fractions were eluted with 70% methanol in 7.5 mM PBS and then dried. After re-dissolving in 2 ml of water, the samples were analyzed by synchronous fluorescence spectroscopy (SFS) (Perkin-Elmer LS50B Luminescence spectrometer, Norwalk, CT, USA) using a wavelength difference of 34 nm (excitation–emission). The recovery of the assay was 82% and the coefficient of variation of the assay was 9%.

### 2.6. Analysis of urinary 1-hydroxypyrene (1-OHP)

The urine samples were immediately frozen (−20 °C) and kept frozen until the laboratory analysis. Urinary 1-OHP was analyzed according to the method developed by Jongeneelen et al. [6]. Briefly, the conjugated metabolite in a urine sample was hydrolysed with β-glucuronidase, and then the sample was measured by high performance liquid chromatography (HPLC) with fluorescence detection. The wavelengths for 1-OHP detection were 242 nm (excitation) and 388 nm (emission). Concentrations of 1-OHP were adjusted for creatinine, and expressed as μmol/mol creatinine.

### 2.7. Aromatic-DNA adducts analysis

The nuclease P1 digested version of the $^{32}$P-post-labeling method [28] was used for the measurement of DNA adducts. For this measurement, 5 μg of DNA was digested with micrococcal nuclease and spleen phosphodiesterase. This hydrolysate was digested by nuclease P1. The digested material was dried and taken up in a total of 2 μl of T4 polynucleotide kinase labeling mixture containing [γ-$^{32}$P] ATP. The labeled samples were spotted and developed on polyethyleneimine-cellulose thin layer chromatography plates (Macherry–Nagel, Düren, Germany) using four solvent systems: D1, 1 M sodium phosphate, pH 6.0; D2, 2.5 M ammonium formate pH 3.5; D3, 3.6 M lithium formate, 8.5 M urea, pH 3.5; D4, 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0; D5, 1.6 M sodium phosphate, pH 6.0. DNA adducts were detected using Bio-Image Analyzer (BAS2000; Fuji Photo Film Co., Tokyo, Japan) after exposing thin layer plates to the Fuji imaging plate. A diagonal radioactive area on the thin layer plates was counted and a background level obtained from the same plate was subtracted. The measurements were performed in duplicate or triplicate for each sample. The results were given as a total number of adducts per $10^8$ normal nucleotides.

### 2.8. Glycophorin A (GPA) assay

For the BR6 GPA assay (BR6), a Becton Dickinson FACScan single-beam flow cytometer (Becton Dickinson Co., San Jose, CA, USA) was used to enumerate cells with the variant NØ or NN phenotypes. BR6 was used together with the antibodies BRIC157 and 6A7 [29]. Blood samples were fixed and immunostained with two fluorescent labeled monoclonal antibodies specific for the M- and N-forms of GPA within 24 h of sample collection. Flow cytometry was used to determine the frequency of erythrocytes that lacked fluorescence from the M-specific antibody. Two variant cell phenotypes were measured simultaneously in the GPA assay. GPA mutation frequency was assessed for only 55 of 190 individuals who provided blood samples due to several factors: (1) the GPA mutation assay can only be performed in half the populations with MN heterozygote; (2) poor sample quality (e.g. hemolysis, not enough amount of blood, etc.).

### 2.9. Determination of genetic polymorphisms

The *GSTM1* and *GSTT1* genotypes were detected as described elsewhere [30]. Briefly, β-globin specific primers were used with *GSTM1* or *GSTT1* specific primers in a multiplex PCR. The absence of

the *GSTM1* or *GSTT1*-specific PCR-product indicated the corresponding null genotype whereas the β-globin specific fragment confirmed proper functioning of the reaction.

### 2.10. Statistical methods

Log-transformed data were used for the statistical analysis after confirming that the biomarker levels were approximately log-normally distributed. The Student's *t*-test was used to compare group means of these biomarkers. Pearson correlation coefficient was used to evaluate correlations among biomarkers. Multiple linear regression analysis was carried out for log-transformed 1-OHPG, 1-OHP, and aromatic-DNA adduct levels. All statistical analyses were performed with SPSS statistical package version 10.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results

Total ambient PAH-levels from the 25 personal samples from the coal tar painters ranged from 0.08 to 22.49 μg/m$^3$ (arithmetic mean = 4.82 ± 5.41 μg/m$^3$; geometric mean = 2.44 ± 3.96 μg/m$^3$). Total ambient PAHs contained 64.1% naphthalene, 11.3% acenaphthene, 6.2% fluorene, 3.9% anthracene, 3.3% pyrene, 2.9% benzo[a]anthracene*, 2.8% fluoranthene, 2.0% acenaphthylene, 0.7% chrysene* and <0.1% benzo[a]pyrene* (*carcinogenic PAHs).

No significant difference in age, sex, alcohol consumption, and smoking habits among the three exposure groups was detected (Table 1).

Urinary 1-OHPG levels were highest in coal tar paint users (mean = 2.24±2.31 μmol/mol creatinine), followed by the general painters (1.38 ± 1.57 μmol/

Table 2
Urinary 1-OHPG levels (μmol/mol creatinine) in relation to exposure, smoking status, green tea consumption and *GSTM1* and *GSTT1* genotypes

| Variables | *N* (208, %) | AM[a] | S.D. | GM[b] | Range | *P*-value |
|---|---|---|---|---|---|---|
| Exposure group | | | | | | |
| On-site control | 23 (11.1) | 0.62 | 0.62 | 0.40 | 0.09–2.16 | <0.001[c] |
| General paint users | 62 (29.8) | 1.38 | 1.57 | 0.99 | 0.19–9.64 | <0.001[c,d] |
| Coal tar paint users | 92 (44.2) | 2.24 | 2.31 | 1.40 | 0.14–13.34 | |
| Missing | 31 (14.9) | | | | | |
| Smoking | | | | | | |
| Non-smokers | 53 (25.5) | 1.14 | 1.00 | 0.79 | 0.09–5.07 | 0.001 |
| Smokers | 122 (58.7) | 1.98 | 2.26 | 1.19 | 0.11–13.34 | |
| Missing | 33 (15.9) | | | | | |
| Green tea | | | | | | |
| Non-drinkers | 132 (63.5) | 1.88 | 2.07 | 1.15 | 0.11–13.34 | 0.002 |
| Drinkers[e] | 43 (20.7) | 1.23 | 1.72 | 0.82 | 0.09–6.13 | |
| Missing | 33 (15.9) | | | | | |
| *GSTM1* | | | | | | |
| Null | 85 (40.9) | 11.80 | 2.03 | 1.12 | 0.12–9.64 | 0.754 |
| Present | 81 (38.9) | 11.62 | 2.03 | 1.03 | 0.09–13.34 | |
| Missing | 42 (20.2) | | | | | |
| *GSTT1* | | | | | | |
| Null | 90 (43.3) | 1.78 | 2.15 | 1.09 | 0.12–13.34 | 0.349 |
| Present | 76 (36.5) | 1.63 | 1.88 | 1.044 | 0.09–9.64 | |
| Missing | 42 (20.2) | | | | | |

[a] Arithmetic mean.
[b] Geometric mean.
[c] Compared with on-site controls.
[d] $P = 0.014$, general paint users vs. coal tar paint users.
[e] Three or four times green tea drinking in a week or a month (150 ml teacup).

mol creatinine). Urinary 1-OHPG levels of both groups of painters were significantly higher than the urinary 1-OHPG levels in on-site controls ($0.62 \pm 0.62$ μmol/mol creatinine) ($P < 0.001$, Student's *t*-test). Urinary 1-OHPG level in those who did not consume of green tea ($1.88 \pm 2.07$ μmol/mol creatinine) was significantly higher than the urinary 1-OHPG level in those who did consume green tea ($1.23 \pm 1.72$ μmol/mol creatinine) ($P = 0.002$, Student's *t*-test) (Table 2).

Urinary 1-OHPG levels were also significantly different between smokers ($n = 122$) and nonsmokers ($n = 53$) ($P = 0.001$, by Student's *t*-test). The number of cigarettes consumed per day showed a dose-response increase in urinary 1-OHPG levels (Pearson's correlation coefficient = 0.206, $P = 0.006$) (data not shown). No significant effect in the 1-OHPG levels were observed for the *GSTM1* and *GSTT1* genotypes (Table 2).

The results of the univariate analyses were further explored in a multivariate regression model (Table 3). Exposure to paints, and smoking were positively associated with urinary 1-OHPG levels, whereas green tea consumption was associated with a significantly reduced 1-OHPG level. Univariate and multivariate analyses with urinary 1-hydroxypyrene levels as the dependent variable showed similar results, as would be expected based on the high correlation between urinary 1-OHPG and 1-hydroxypyrene levels (Pearson's correlation coefficient = 0.81, $P < 0.001$, $n = 175$) (Fig. 1).

Table 3
Predictors of urinary 1-OHPG levels by multiple linear regression

| Variables | β | S.E. | *P*-value | Overall model ($R^2$) |
|---|---|---|---|---|
| Intercept | −0.51 | 0.29 | | 0.24 |
| Exposure[a] | | | | |
| General paint users | 0.86 | 0.21 | <0.001 | |
| Coal tar paint users | 1.22 | 0.20 | <0.001 | |
| Smoking (yes vs. no) | 0.32 | 0.14 | 0.028 | |
| Green tea (yes vs. no) | −0.47 | 0.15 | 0.003 | |

[a] Compared to on-site controls.

Aromatic-DNA adduct levels tended to be higher in coal tar paint users ($P = 0.06$) and painters ($P = 0.07$) compared to on-site controls (Table 4). No differences in adduct levels were observed, between the two groups of painters, and the combined group showed greater adduct levels than on-site controls ($P = 0.05$). Smoking and *GSTM1* and *GSTT1* genotypes were not significantly associated with aromatic-DNA adduct levels.

No significant difference was observed in GPA variant frequency between the three study groups (Fig. 2). Furthermore, no significant correlation was observed between urinary 1-OHPG levels and either

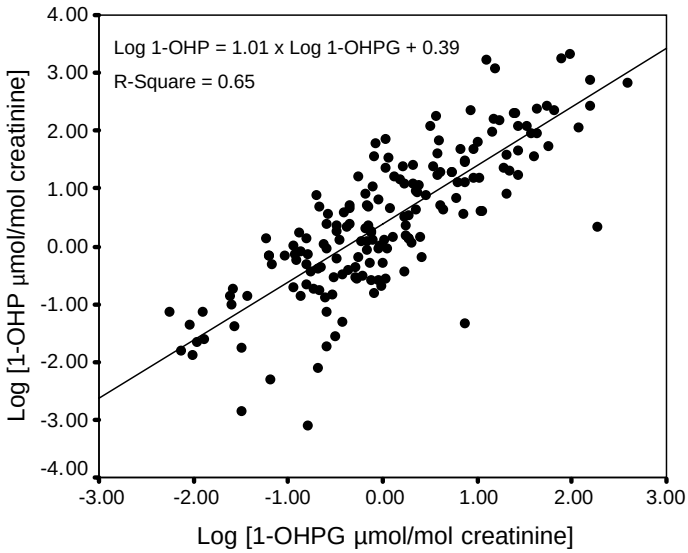


Fig. 1. Correlation of 1-hydroxypyrene (1-OHP) with 1-hydroxypyrene glucuronide (1-OHPG), $P < 0.01$, $n = 175$.

Table 4
Aromatic-DNA adducts/$10^8$ nucleotides in relation to exposure, smoking status and *GSTM1* and *GSTT1* genotypes

| Variables | *N* (208, %) | AM[a] | S.D. | GM[b] | Range | *P*-value |
|---|---|---|---|---|---|---|
| Exposure groups | | | | | | |
| On-site control | 17 (8.2) | 0.26 | 0.13 | 0.24 | 0.12–0.61 | |
| General paint users | 59 (28.4) | 0.38 | 0.24 | 0.32 | 0.07–1.35 | 0.061[c] |
| Coal tar paint users | 74 (35.6) | 0.38 | 0.23 | 0.31 | 0.08–1.14 | 0.070[c,d] |
| Missing | 58 (27.9) | | | | | |
| Smoking | | | | | | |
| Non-smokers | 40 (19.2) | 0.37 | 0.26 | 0.30 | 0.12–1.35 | 0.809 |
| Smokers | 107 (51.4) | 0.36 | 0.22 | 0.31 | 0.07–1.14 | |
| Missing | 61 (29.3) | | | | | |
| GSTM1 | | | | | | |
| Null | 81 (38.9) | 0.35 | 0.20 | 0.29 | 0.07–1.02 | 0.399 |
| Present | 69 (33.2) | 0.39 | 0.26 | 0.32 | 0.08–1.35 | |
| Missing | 58 (27.9) | | | | | |
| GSTT1 | | | | | | |
| Null | 84 (40.4) | 0.36 | 0.24 | 0.30 | 0.08–1.35 | 0.971 |
| Present | 66 (31.7) | 0.37 | 0.22 | 0.31 | 0.07–1.11 | |
| Missing | 58 (27.9) | | | | | |

[a] Arithmetic mean.
[b] Geometric mean.
[c] Compared to on-site controls.
[d] $P = 0.896$, general paint users vs. coal tar paint users.

aromatic-DNA adducts ($R = 0.11$) or GPA mutation frequencies (NØ, $R = -0.21$; NN, $R = 0.03$).

## 4. Discussion

In this study, urinary 1-OHPG levels were significantly higher in painters including both coal tar paint users and general paint users than in an on-site non-exposed control group. Furthermore, the urinary 1-OHPG levels in coal tar users were higher than in general paint users.

Our findings of higher levels of urinary 1-OHPG in general paint users than in controls might be due to several factors. Since general paint users usually work closely with coal paint users, they may be exposed to elevated ambient levels of PAHs. In addition, paints not officially labeled as coal tar paints (based on Material Safety Data Sheets) contain significant amounts of PAHs.

Urinary 1-OHPG and 1-OHP levels were significantly inversely associated with green tea consumption. Green tea contains various beneficial compounds, particularly (−)-epigallocatechin gallate (EGCG), that have been shown to be protective against cancer and mutations [30]. EGCG is a polyphenol with strong antioxidant properties that has also been shown to lower the activity levels of several drug-metabolizing enzymes, including cytochrome P450 (CYP)1A1 which is presumably involved in the metabolism of pyrene to 1-hydroxypyrene [31]. Several studies demonstrated that green tea constituents (i.e. EGCG) inhibited the metabolic activation of benzo[a]pyrene by both mouse and human CYPs (i.e. CYP1A1, CYP1A2 and CYP3A4) [32–34].

Although 1-OHP has been widely applied to biomonitoring of PAH exposure, urinary 1-OHPG was shown to be a better biomarker for medium to low level exposure to PAHs; urinary 1-OHPG exhibits three- to five-fold more fluorescence than 1-OHP. In addition 1-OHPG levels correlated well with urinary 1-OHP levels (Fig. 1).

The finding that aromatic-DNA adduct levels in painters were marginally higher than in on-site controls but not related to PAH exposure suggests that paints used in this shipyard may contain significant amounts of genotoxic aromatic compounds other than PAHs.

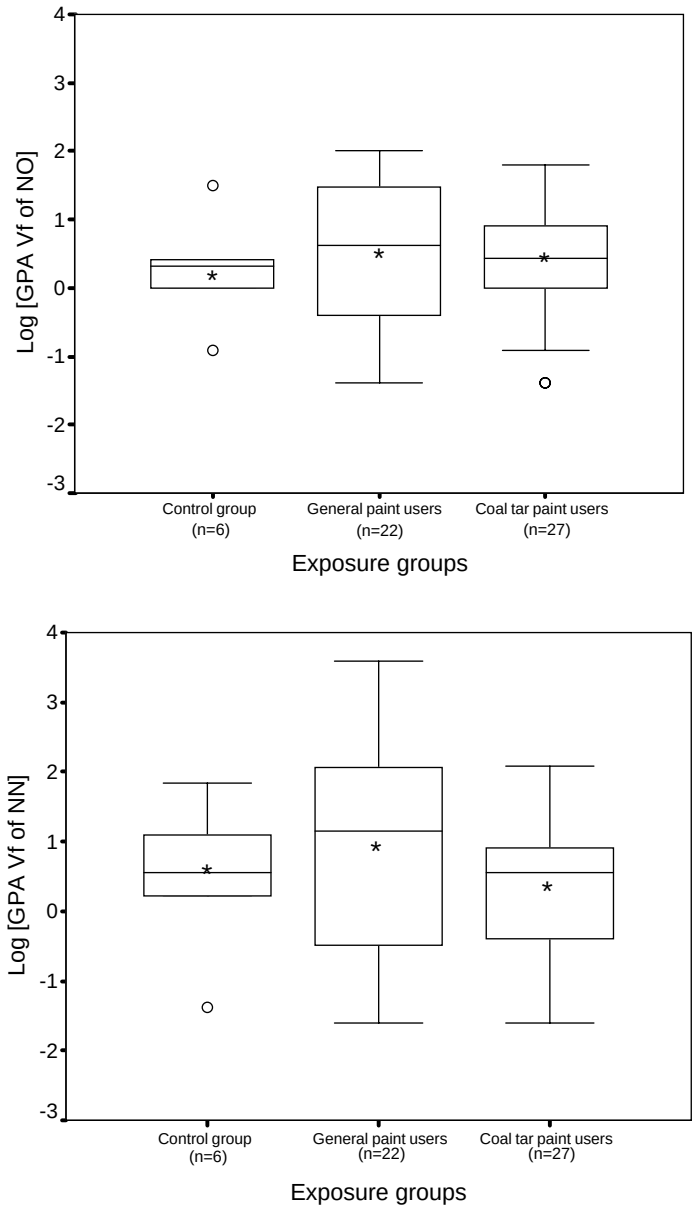


Fig. 2. Box plot of log(GPA variant frequencies of NO or NN) (per $10^6$ erythrocyte cells). Boxes encompass the 25th–75th percentiles; middle lines the medians; (★) mean; whiskers the 10th–90th percentiles; (○) represent values beyond the 90th percentiles.

In present study, no significant association between *GSTM1* and *GSTT1* genotypes and urinary 1-OHPG levels or aromatic adducts was observed in this study. This is generally consistent with other studies reporting no association between *GSTM1* genotype and DNA or protein adducts [20,21]. Contrary to our observations, however, some studies have shown a moderate effect of *GSTM1* null genotype on aromatic-DNA adducts or 1-OHP levels [18,19,35]. Inconsistent results from previous studies of the genetic polymorphism of *GSTM1* and *GSTT1* on the levels of urinary PAH metabolites or aromatic-DNA

adducts in peripheral blood might be due to several factors: (1) different levels of exposure; (2) different routes of exposure; (3) inter-laboratory difference in quantifying methods [36]. Future well-designed epidemiological studies are therefore, needed to evaluate the role of *GSTM1* and *GSTT1* in pyrene metabolism.

The GPA mutation assay has been used to determine genotoxic effects on bone marrow in populations exposed to ionizing radiation [37]. The GPA locus somatic cell mutation assay showed persistent increases in NØ variant frequency 40 years after exposure in survivors of the atomic bomb at Hiroshima [38,39]. Although the trend appears to indicate an increased level of GPA NØ in the exposed group, we found no statistically significant increase in GPA mutation frequency in individuals working in shipyard painting areas, consistent with a recent report on GPA mutation frequency in relation to exposure to butadiene, a known genotoxic compound [24,25]. However, given the small control group ($n = 6$) interpretation of these results have to be interpreted with caution.

In conclusion, These results suggest that painters in the shipyard were exposed to significant amounts of PAHs and possibly to other genotoxic aromatic compounds, and that urinary 1-OHPG may be a potential biomarker of PAH exposure in this population. Given the elevated 1-OHPG and DNA-adduct levels found at this shipyard, control measures focused on both the dermal and respiratory route should be implemented until these hazardous paints can be fazed out.

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## References

[1] L.V. Cralley, P.F. Woolrich, J.E. Mutchler, K.J. Caplan, In-plant Practices for Job Related Health Hazards Control, Wiley, New York, 1989, pp. 457−485.

[2] Y.C. Sin, K.Y. Lee, Chemical composition of painting materials used in some Korean shipyards, Korean Ind. Hyg. Assoc. J. 9 (1999) 156–172.

[3] K. Steenland, S. Palu, Cohort mortality study of 57,000 painters and other union members: a 15-year update, Occup. Environ. Med. 56 (1999) 315–321.

[4] R. Chen, A. Seaton, A meta-analysis of painting exposure and cancer mortality, Cancer Detect. Prev. 22 (1998) 533–539.

[5] http://home.kosha.net/~cfodr/main_2.htm.

[6] F.J. Jongeneelen, R.B.M. Arizon, P.T. Henderson, Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine, J. Chromatogr. 413 (1987) 227–232.

[7] P.T. Strickland, D. Kang, E.D. Bowman, A. Fitzwilliam, T.E. Downing, N. Rothman, J.D. Groopman, A. Weston, Identification of 1-hydroxypyrene glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas chromatograpy mass spectrometry, Carcinogenesis 15 (1994) 483–487.

[8] P.T. Strickland, D. Kang, Urinary 1-hydroxypyrene and other PAH metabolites as biomarkers of exposure to environmental PAH in air particulate matter, Toxicol. Lett. 108 (1999) 191–199.

[9] R. Singh, M. Tucek, K. Maxa, J. Tenglerova, E.H. Weyand, A rapid and simple method for the analysis of 1-hydroxypyrene glucuronide: a potential biomarker for polycyclic aromatic hydrocarbon exposure, Carcinogenesis 16 (1995) 2909–2915.

[10] B. Coles, B. Ketterer, The role of glutathione and glutathione transferases in chemical carcinogenesis, Critic. Rev. Biochem. Mol. Biol. 25 (1990) 47–70.

[11] B. Ketterer, J.M. Harris, G. Talaska, D.J. Meyer, S.E. Pemble, J.B. Taylor, N.P. Lang, F.F. Kadlubar, The human glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer, Environ. Health Perspect. 98 (1992) 87–94.

[12] S. Pemble, K.R. Schroeder, S.R. Spencer, D.J. Meyer, E. Hallier, H.M. Bolt, B. Ketterer, J.B. Taylor, Human glutathione *S*-transferase theta (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism, Biochem. J. 300 (1994) 271–276.

[13] A.K. Alexandrie, M. Warholm, U. Carstensen, A. Axmon, L. Hagmar, J.O. Levin, C. Ostman, A. Rannug, CYP1A1 and *GSTM1* polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure, Carcinogenesis 21 (2000) 669–676.

[14] G. Gabbani, S.M. Hou, B. Nardini, M. Marchioro, B. Lambert, E. Clonfero, *GSTM1* and NAT2 genotypes and urinary mutagens in coke oven workers, Carcinogenesis 17 (1996) 1677–1681.

[15] S. Øvrebø, D. Ryberg, A. Haugen, H.L. Leira, Glutathione *S*-transferase M1 and P1 genotypes and urinary excretion of 1-hydroxypyrene in coke oven workers, Sci. Total Environ. 220 (1998) 25–31.

[16] Y.C. Hong, J.H. Lee, H.S. Park, K.H. Lee, S.J. Lee, C.K. Lee, D. Kang, Variations in urinary 1-hydroxypyrene glucuronide in relation to smoking and the modification effects of *GSTM1* and *GSTT1*, Toxicol. Lett. 108 (1999) 217–223.

[17] P.V. Nerurkar, L. Okinaka, C. Aoki, A. Seifried, A. Lum-Jones, L.R. Wilkens, L. Le Marchand, CYP1A1, *GSTM1*, *GSTT1* genetic polymorphisms a urinary 1-hydroxypyrene excretion in non-occupationally exposed individuals, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 1119–1122.

[18] M. Rojas, I. Cascorbi, K. Alexandrov, E. Kriek, G. Auburtin, L. Mayer, A. Kopp-Schneider, I. Roots, H. Bartsch, Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, *GSTM1* and *GSTT1* polymorphisms, Carcinogenesis 21 (2000) 35–41.
[19] D. Butkiewicz, E. Grzybowska, D.H. Phillips, K. Hemminki, M. Chorazy, Polymorphisms of the *GSTP1* and *GSTM1* genes and PAH-DNA adducts in human mononuclear white blood cells, Environ. Mol. Mutagen. 35 (2000) 99–105.
[20] K. Hemminki, C. Dickey, S. Karlsson, D. Bell, Y. Hsu, W.Y. Tsai, L.A. Mooney, K. Savela, FP. Perera, Aromatic-DNA adducts in foundry workers in relation to exposure, Carcinogenesis 18 (1997) 345–350.
[21] M.C. Poirier, A. Weston, B. Schoket, H. Shamkhani, C.F. Pan, M.A. McDiarmid, B.G. Scott, D.P. Deeter, J.M. Heller, D. Jacobson-Kram, N. Rothman, Biomonitoring of United States Army soldiers serving in Kuwait in 1991, Cancer Epidemiol. Biomarkers Preven. 7 (1998) 545–551.
[22] R.G. Langlois, W.L. Bigbee, R.H. Jensen, Measurements of the frequency of human erythrocytes with gene expression loss phenotypes at the glycophorin A locus, Hum. Genet. 74 (1986) 353–362.
[23] S.G. Grant, W.L. Bigbee, In vivo somatic mutation and segregation at the human glycophorin A (GPA) locus: phenotypic variation encompassing both gene-specific and chromosomal mechanisms, Mutat. Res. 288 (1993) 163–172.
[24] R.B. Hayes, L. Zhang, J.A. Swenberg, S.N. Yin, L. Xi, J. Wiencke, W.E. Bechtold, M. Yao, N. Rothman, R. Haas, J.P. O'Neill, J. Wiemels, M. Dosemeci, G. Li, M.T. Smith, Markers for carcinogenicity among butadiene-polymer workers in China, Chem. Biol. Interact. 135–136 (2001) 455–464.
[25] R.B. Hayes, L. Zhang, S. Yin, J.A. Swenberg, L. Xi, J. Wiencke, W.E. Bechtold, M. Yao, N. Rothman, R. Haas, J.P. O'Neill, D. Zhang, J. Wiemels, M. Dosemeci, G. Li, M.T. Smith, Genotoxic markers among butadiene polymer workers in China, Carcinogenesis 21 (2000) 55–62.
[26] National Institute for Occupational Safety and Health (NIOSH), NOISH Manual of Analytical Methods (NMAM) 5515: Ploynuclear Aromatic hydrocarbons by GC, fourth ed., 1994.
[27] G. Mastrangelo, E. Fadda, V. Marzia, Polycyclic aromatic hydrocarbons and cancer in man, Environ. Health Perspect. 104 (1996) 1166–1170.
[28] M. Ichiba, L. Hagmar, A. Rannug, B. Hogstedt, A.-K. Alexandrie, U. Carstensen, K. Hemminki, Aromatic-DNA adducts micronuclei and genetic polymorphism for CYP1A1 and *GSTT1* in chimney sweeps, Carcinogenesis 15 (1994) 1347–1352.
[29] R.G. Langlois, B.A. Nisbet, WL. Bigbee, D.N. Ridinger, R.H. Jensen, An improved flow cytometric assay for somatic mutations at the glycophorin A locus in human, Cytometry 11 (1990) 513–521.
[30] S. K Park, K.Y. Yoo, S.J. Lee, S.U. Kim, S.H. Ahn, D.Y. Noh, K.J. Choe, P.T. Strickland, A. Hirvonen, D. Kang, Alcohol consumption, glutathione *S*-transferase M1 and T1 genetic polymorphisms and breast cancer risk, Pharmacogenetics 10 (2000) 301–309.
[31] Z.Y. Wang, M. Das, D.R. Bickers, H. Mukhtar, Interaction of epicatechins derived from green tea with rat hepatic cytochrome P-450, Drug Metab. Dispos. 16 (1988) 98–103.
[32] T. Jiang, B.W. Glickman, J.G. deBoer, Protective effect of green tea against benzo[a]pyrene-induced mutations in the liver of Big Blue transgenic mice, Mutat. Res. 480–481 (2001) 147–151.
[33] S. Muto, K. Fujita, Y. Yamazaki, T. Kamataki, Inhibition by green tea catechins of metabolic activation of procarcinogens by human cytochrome P450, Mutat. Res. 479 (2001) 197–206.
[34] S.G. Khan, S.K. Katiyar, R. Agarwal, H. Mukhtar, Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention, Cancer Res. 52 (1992) 4050–4052.
[35] H.M. Nan, H. Kim, H.S. Lim, J.K. Choi, T. Kawamoto, J.W. Kang, C.H. Lee, Y.D. Kim, E.H. Kwon, Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, *GSTM1* and *GSTT1* on urinary 1-hydroxypyrene and 2-naphthol concentrations, Carcinogenesis 22 (2001) 787–793.
[36] H. Autrup, Genetic polymorphisms in human xenobiotics metabolizing enzymes as susceptibility factors in toxic response, Mutat. Res. 464 (2000) 65–76.
[37] RH. Jensen, R.G. Langlois, W.L. Bigbee, S.G. Grant, M. Pilinskaya II, I. Vorobtsova, P. Pleshanov, Elevated frequency of glycophorin A mutations in erythrocytes from Chernobyl accident victim, Radiat. Res. 141 (1995) 129–135.
[38] R.G. Langlois, W.L. Bigbee, S. Kyoizumi, N. Nakamura, M.A. Bean, M. Akiyama, R.H. Jensen, Evidence for an increased frequency of somatic cell mutations at the glycophorin A locus in A-bomb survivors, Science 236 (1987) 445–448.
[39] R.G. Langlois, M. Akiyama, Y. Kusunoki, W.L. Bigbee, S.G. Grant, B.R. Du Pont, R.H. Jensen, Analysis of somatic mutations at the glycophorin A locus in atomic bomb survivors: a comparative study of assay methods, Radiat. Res. 136 (1993) 111–117.